

Title: API 20E Microbial Identification SOP

Approvals:

Preparer: Melanie Lenahan _____ Date: 052907 _____
Reviewer: Martha Salas _____ Date: 052907 _____

1. Purpose:

1.1. To perform a microbial identification assay.

2. Scope:

2.1. This procedure is intended as a standardized identification system for *Enterobacteriaceae* and non-fastidious Gram-negative rods included in the database.

3. Responsibilities:

- 3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
- 3.2. It is the responsibility of the students to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

- 4.1. API 20E System Brochure
- 4.2. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – cGMP (FDA publication, September 2004)
- 4.3. United States Pharmacopeia 25
- 4.4. Gram Stain SOP
- 4.5. Bergey's Manual of Systematic Bacteriology

5. Definitions:

5.1. *Enterobacteriaceae*: Family of Gram-negative, rod bacteria that inhabit soil, water and are commonly found in the large bowel of humans. Most common organisms isolated from clinical specimens.

6. **Precautions:** Aseptic technique and standard precautions for handling microbial cultures.

7. Materials:

- 7.1. API 20E System strip, incubator tray and cover
- 7.2. API 20E Results sheet
- 7.3. API 20E Quick Index Booklet or the API 20E Profile Recognition System
- 7.4. Test tube rack
- 7.5. Disposable gloves
- 7.6. Bacterial cultures including E. coli control (ATCC #25922)
- 7.7. 10% ferric chloride
- 7.8. 3% hydrogen peroxide
- 7.10 McFarland No. 0.5 standard
- 7.11. Nitrate reagents (I&II)
- 7.12. Oxidase
- 7.13. Zn dust
- 7.14. Kovac's reagent
- 7.15. VPI and VP2 reagents
- 7.16. Sterile mineral oil
- 7.17. Bacterial incubator set at 36°C
- 7.18. Test-tube rack

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8. Procedure:

- 8.1. Using the Gram-stain technique (Gram-stain SOP) determine that the bacterial culture is a Gram-negative rod.
- 8.2. **Oxidase test:**
 - 8.2.1. Using aseptic technique select an isolated colony from the streak plate.
 - 8.2.2. Smear a small amount of the colony over a small area of filter paper.
 - 8.2.3. Perform the oxidase test according to the manufacturer's instructions. Record the result on the API 20E Results sheet (21st identification test).
- 8.3. **Preparation of the inoculum:**
 - 8.3.1. Open an ampule of API Suspension Medium (5 ml).
 - 8.3.2. Remove a well-isolated colony from the streak plate and transfer to the API Suspension Medium. Mix to emulsify and obtain a homogenous suspension.
 - 8.3.3. Check the turbidity of the suspension to that of the McFarland No. 0.5 standard. If necessary, add more bacteria.
 - 8.3.4. The suspension must be used promptly after preparation.
- 8.4. **Preparation of the API strip:**
 - 8.4.1. Obtain an incubation box (tray and lid).
 - 8.4.2. Add 5 ml of distilled water to the bottom of the incubation tray.
 - 8.4.3. Record your name, date and strain reference on the elongated flap of the incubation tray.
 - 8.4.4. Remove the strip from the sealed pouch and place it in the incubation tray.
- 8.5. **Inoculation of the API strip:**
 - 8.5.1. Gently shake the 5 ml of bacterial suspension.
 - 8.5.2. Remove the cap and fill the 5-ml Pasteur pipette with the bacterial suspension.
 - 8.5.3. Tilt the incubation strip to avoid forming air bubbles.
 - 8.5.4. Using the Pasteur pipette, fill both the tube and the cupule of the tests CIT, VP and GEL.
 - 8.5.5. Fill only the tube (NOT the cupule) of the other tests.
 - 8.5.6. Overlay mineral oil and completely fill the cupule section of the tests ADH, LDC, ODC, H₂S and URE.
 - 8.5.7. Place the lid on the incubation tray and incubate at 36°C ± 2°C for 18-24 hours.
 - 8.5.8. Make an isolation streak on a TSA plate with a portion of the bacterial suspension to ascertain the purity of the sample.
- 8.6. **Reading the API strip:**
 - 8.6.1. After the incubation period, read the strip by referring to the Reading Table.
 - 8.6.2. If **3 or more tests** are positive, record all reactions not requiring the addition of reagents (do NOT read TDA, VP, IND). Record the results by placing a (+) for a positive reaction and a (-) for a negative reaction and continue with step 8.6.3. If the number of positive tests is **less than 3** reincubate the strip for a further 24 hours (± 2 hours) before continuing to step 8.6.3
 - 8.6.3. Reveal the tests which require the addition of reagents and add the following test reagents in the order listed. In all cases, read the results immediately after adding the reagents and waiting the proper length of time. Do not replace the lid on the tray until all results have been collected. Record results.

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- 8.6.3.1. **TDA test:** Add 1 drop of 10% ferric chloride to the TDA tube. A reddish brown color indicates a positive reaction. A negative reaction is yellow.
- 8.6.3.2. **VP test:** Add 1 drop each of VP1(40% KOH) and VP2(α -naphthol) solutions to the VP microtube. The KOH solution should be added first. Wait at least 10 minutes. A pink color developed in the whole cupule indicates a positive reaction. No color change is a negative reaction.
- 8.6.3.3. **IND test:** This test must be performed last. Add 1 drop of Kovac's/James reagent to the IND tube. A positive test is indicated by a red ring with 2 minutes. A yellow ring is a negative reaction.

8.7. Interpretation:

- 8.7.1. Identification is determined by the numerical profile.
- 8.7.2. On the result sheet, the tests are separated into groups of 3 and a value of 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number is obtained for the 20 tests of the API 20 E strip. The oxidase reaction constitutes the 21st test and has a value of 4 if it is positive.
- 8.7.3. Identify the microorganism by entering the 7-digit numerical profile manually using the APIweb software.
- 8.7.4. Print out and record your results.
- 8.7.5. If the 7-digit profile is not discriminatory enough perform the following supplementary tests.
 - 8.7.5.1. Reduction of nitrates to nitrites (NO₂) and N₂ gas: Add 1 drop each of NIT 1 and NIT2 to the GLU tube. Wait 2 to 5 minutes. A red color indicates a positive reaction (NO₂). A negative reaction (yellow) may be due to the reduction to nitrogen. Add 2 to 3 mg of Zn dust to the GLU tube. After 5 minutes, if the tube remains yellow this indicates a positive reaction (N₂). If the test turns orange-red, this is a negative reaction.
 - 8.7.5.2. Identify the microorganism by entering the 9-digit numerical profile manually using the APIweb software.

9. History:

- 9.1. Melanie Lenahan, 052907, initial release
- 9.2. Sheila Byrne, 052208, revision 1
- 9.3. Sheila Byrne, 071910, revision 2
- 9.4. Sheila Byrne, 063014 revision 3, SOP name change

TABLE OF RESULTS					
#	Test	Substrate/Activity	Result	Interpretation	Symbol
1	ONPG	O-nitrophenyl- β -D-galactopyranoside	Yellow ¹	Organism produces beta-galactosidase	+
			Colorless	Organism does not produce beta-galactosidase	-
2	ADH	Arginine	Red/Orange	Organism produces arginine dehydrogenase	+
			Yellow	Organism does not produce arginine dehydrogenase	-
3	LDC	Lysine	Red/Orange ²	Organism produces lysine decarboxylase	+
			Yellow	Organism does not produce lysine decarboxylase	-
4	ODC	Ornithine	Red/Orange ²	Organism produces ornithine decarboxylase	+
			Yellow	Organism does not produce ornithine decarboxylase	-
5	CIT	Sodium citrate	Blue-green/Blue ³	Organism utilizes citrate as sole carbon source	+
			Pale green/Yellow	Organism does not utilize citrate	-
6	H ₂ S	Sodium thiosulfate	Black	Organism reduces sulfur	+
			Colorless/grayish	Organism does not reduce sulfur	-
7	URE	Urea	Red/Orange ²	Organism produces urease	+
			Yellow	Organism does not produce urease	-
8	TDA	Tryptophan	Brown-red	Organism produces tryptophan deaminase	+
			Yellow	Organism does not produce tryptophan deaminase	-
9	IND	Tryptophane	Red ring	Organism produces indole	+
			Yellow	Organism does not produce indole	-
10	VP	Creatine Sodium pyruvate	Pink/red ⁴	Organism produces acetoin	+
			Colorless	Organism does not produce acetoin	-
11	GEL	Kohn's charcoal gelatin	Diffusion of black pigment	Organism produces gelatinase	+
			No black pig- ment diffusion	Organism does not produce gelatinase	-
12	GLU	Glucose ⁵	Yellow	Organism ferments glucose	+
			Blue/blue-green	Organism does not ferment glucose	-
13	MAN	Mannitol ⁵	Yellow	Organism ferments mannitol	+
			Blue/blue-green	Organism does not ferment mannitol	-
14	INO	Inositol ⁵	Yellow	Organism ferments inositol	+
			Blue/blue-green	Organism does not ferment inositol	-
15	SOR	Sorbitol ⁵	Yellow	Organism ferments sorbitol	+
			Blue/blue-green	Organism does not ferment sorbitol	-

¹ A very pale yellow is also positive.² Orange after 36 hours is negative.³ Reading made in the cupule.⁴ A slightly pink color after 5 minutes is negative.⁵ Fermentation begins in the lower portion of the tube; oxidation begins in the cupule.

TABLE 5-34 API 20 E results and interpretations.

TABLE OF RESULTS

#	Test	Substrate/Activity	Result	Interpretation	Symbol
16	RHA	Rhamnose ^s	Yellow	Organism ferments rhamnose	+
			Blue/blue-green	Organism does not ferment rhamnose	-
17	SAC	Sucrose (saccharose) ^s	Yellow	Organism ferments sucrose	+
			Blue/blue-green	Organism does not ferment sucrose	-
18	MEL	Melibiose ^s	Yellow	Organism ferments melibiose	+
			Blue/blue-green	Organism does not ferment melibiose	-
19	AMY	Amygdalin ^s	Yellow	Organism ferments amygdalin	+
			Blue/blue-green	Organism does not ferment amygdalin	-
20	ARA	Arabinose ^s	Yellow	Organism ferments arabinose	+
			Blue/blue-green	Organism does not ferment arabinose	-
21	OX	Separate test done on paper test strip	Violet	Organism possesses cytochrome-oxidase	+
			Colorless	Organism does not possess cytochrome-oxidase	-
22	GLU (nitrate reduction)	Potassium nitrate	Red after addition of reagents	Organism reduces nitrate to nitrite	+
			Yellow after addition of reagents	Organism does not reduce nitrate to nitrite	-
			Yellow after addition of zinc	Organism reduces nitrate to N ₂ gas	+
			Orange-red after addition of zinc	Organism does not reduce nitrate to N ₂ gas	-
23	MOB	Motility Medium or wet mount slide	Motility	Organism is motile	+
			Nonmotility	Organism is not motile	-
24	McC	MacConkey Medium	Growth	Organism is probably Enterobacteriaceae	+
			No growth	Organism is not Enterobacteriaceae	-
25	OF	Glucose	Yellow under mineral oil	Organism ferments glucose	+
			Green under mineral oil	Organism does not ferment glucose (not <i>Enterobacteriaceae</i>)	-
			Yellow without mineral oil	Organism either ferments glucose or utilizes it oxidatively	+
			Green without mineral oil	Organism does not utilize glucose (not <i>Enterobacteriaceae</i>)	-

TABLE 5-34 (Continued).